HUMAN INTESTINAL DENDRITIC CELLS DICTATE INFLAMMATION AND T-CELL POLARIZATION

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*Introduction:*Enormous diversity of commensal bacteria determines individual functions acting on the development and activities of the human immune system. This complexity can directly be translated to T-cell polarization to support tolerance induction or inflammation. We have establisheda sensitive *in vitro* culture system for investigating the response of monocyte-derived dendritic cell (moDC) subsets to various gutbacteria by monitoring the expression of type I/II CD1 proteins, secretion of chemokines, pro-inflammatory and T-cell polarizing cytokinesin the context of the T-cell polarizing potential of these moDC subsets. Under physiological conditions the gut microenvironment is conditioned by all-transretinoic acid (ATRA) produced by gutepithelial cells and $CD103^+$ DCs. To consider the impact of this special microenvironment on moDC-induced T-cellresponses we compared the effects of selected microbes on DC and T-celldevelopment in the absence and presence of ATRA.
*Methods:*Monocytes were separated from human buffy coats and differentiated *in vitro*in the presence of IL-4 and GM-CSF with or without 1 nM ATRA for 2 days. Gram(-) (*Schaedler'sE. coli, E.coli 058, M. morganii)* and Gram(+) *(B.subtilis)*bacteria were grown in antibiotic-free LB medium and were added to the 2-day moDCs for 24 hrs.Activation of moDCwas monitored by the expression of membrane CD1a, CD1d and CD83 by FACS analysis. Culture supernatants of activated moDCwere collected on day 3 and cytokine concentrations were determined by ELISA. The number of IFNγ and IL-17 producing T-cells was measured by ELISPOT assay. Expression levels of selected NOD-like receptors and genes involved in ATRA synthesisweremeasured by qRT-PCR.
*Results:*Increased expression of CD83 revealed that all tested commensal bacteriawere able to activated moDCsfor pro- and anti-inflammatory cytokine secretion. ATRA had a significant impact on the differentiation, inflammatory response and T-cell polarizing activity of moDCs. It increased the cell surface expression of CD1a while increased that of CD1d, previously shown to be associated with a shift in moDC functionality. ATRA also enhancedIL-1ß secretion and upregulated the expression of genesinvolvedin ATRA synthesisandNLRP12mRNAlevels. Interestingly, theseATRA-inducedeffectscould be counterregulatedbythe tested microbe. The interaction of microbes resulted in IL-23 production supporting Th17 polarization of autologous T-cellsandincreased the number ofIFNγ producing T-cells however, these effects were down modulated by ATRA.
*Discussion:*In our culture system we identified two moDC subpopulations referred as DC-$SIGN^+CD11c^+CD14^{med}CD1a^+CD1d^-$ and DC-$SIGN^+CD11c^+CD14^+CD1a^-CD1d^+$ cells, which respond to and coordinateof stimuli from commensal bacteria differently to induce T-cell polarization and expansion. Our results also showed that the tested bacteria modulate the differentiation and activation of moDCs in a dose- and bacterial strain-dependent manner. Moreover, the interplay of moDC and commensals can be modified by the actual milieu of the cells such as ATRA.